

ART 3A AGENT

Claims

- 1) A method for determining the sequence of a nucleic acid molecule comprising the steps of;
 - 5 a) providing a single-stranded form of said nucleic acid molecule;
 - b) hybridizing a primer to said single stranded form of said nucleic acid molecule to form a template/primer complex;
 - c) enzymatically extending the primer by the addition of a polymerase and a mixture of at least one nucleotide and at least one labeled derivative of the at least one nucleotide, wherein the at least one labeled derivative of the at least one nucleotide comprises a label linked to the nucleotide via a cleavable link and wherein the amount of labeled derivative of the at least one nucleotide in said mixture of the at least one nucleotide and the labeled derivative of the at least one nucleotide is within the range of 1-50 mole-%, 10 1-40 mole-%, 1-30 mole-%, or 1-20 mole-%.
 - 15 d) determining the type of nucleotide added to the primer; and
 - e) repeating steps c) to d) at least once.
- 2) A method according to claim 1, in which the amount of labelled derivative of the at least one nucleotide in said mixture is within the range of 5-50 mole-%, 5-40 mole-%, 5-30 mole-%, or 5-20 mole-%.
- 20 3) A method according to claim 1, in which the amount of labelled derivative of the at least one nucleotide in said mixture is within the range of 10-50 mole-%, 10-40 mole-%, 10-30 mole-%, or 10-20 mole-%.
- 25 4) A method according to any one of claims 1 – 3, wherein the single-stranded form of said nucleic acid molecule is attached to a carrier.
- 5) A method according to claim 4, wherein the means for attachment is selected from the group of: a) specific binding to a hydrophobic compound, 30 an oligonucleotide, an antibody or a fragment thereof, a protein, a peptide,

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- an intercalating agent, biotin, streptavidin or avidin; or b) covalent coupling using an amino-linker and an epoxy-treated carrier.
- 6) A method according to claim 4, wherein the carrier is selected from the group of a gel, a solid or porous bead, a surface or a fiber.
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- 7) A method according to any one of claims 1 - 3, in which the label is neutralized after step d) by the addition of a label-interacting agent or by bleaching.
- 10 8) A method according to claim 7, in which the bleaching is performed by photo-bleaching.
- 9) A method according to claim 1-3 in which the link between the incorporated nucleotide and the label is cleaved after step d).
- 15 10) A method according to claim 1-3, in which the link between the fluorophore and nucleotide is a disulfide bond.
- 11) A method according to claim 10 in which the cleavage is performed by the addition of a reducing agent, thereby exposing a thiol group.
- 20 12) A method according to claims 10 or 11, in which the exposed thiol group is capped by a suitable reagent, such as iodoacetamide or N-ethylmaleimide.
- 13) A method according to any of the claims above in which the linker between the disulfide bridge and the base is shorter than 8 atoms.
- 25 14) A method according to any of the above claims in which the step c) is performed at a pH below 7, preferably at a pH below 6.5, or more preferably at a pH below 6.
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15) A method according to any of the above claims, in which the derivative of said nucleotide is a dideoxynucleotide or an acyclic nucleotide analog.

16) A method according to any of the above claims, in which an agent chosen

5 from the group comprising the following; alkaline phosphatase, PPi-ase, apyrase, dimethylsulfoxide, polyethylene glycol, polyvinylpyrrolidone, spermidine, detergents such as NP-40, Tween 20 and Triton X-100; various proteins that affect secondary structure of DNA including Single Stranded DNA Binding Protein (SSB) or the protein of Gene 32, is added.

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17) A kit comprising, in separate compartments, a mixture of at least one nucleotide and at least one labelled derivative of the at least one nucleotide, wherein the at least one labeled derivative of the at least one nucleotide comprises a label linked to the nucleotide via a cleavable link and wherein the amount of labeled derivative of the at least one nucleotide in said mixture of the at least one nucleotide and the labeled derivative of the at least one nucleotide is within the range of 1-50 mole-%, 1-40 mole-%, 1-30 mole-%, or 1-20 mole-%, preferably in the range of 5-20 mole-%, 5-30 mole-%, 5-40 mole-% or 5-50 mole-%, and even more preferably in the range of 10-20 mole-%, 10-30 mole-%, 10-40 mole-% or 10-50 mole-%, and a reducing agent.

18) A kit according to claim 17 further comprising at least one of the following components; a DNA polymerase, a carrier, a capping agent, an apyrase, an alkaline phosphatase, a PPi-ase, a single strand binding protein or the protein of Gene 32, for performing the method according to any of the claims 1-14.

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